α -Pyrone derivatives, tetra/hexahydroxanthones, and cyclodepsipeptides from two freshwater fungi

Tamam El-Elimat^a, Huzefa A. Raja^b, Cynthia S. Day^c, Hana McFeeters^d, Robert L. McFeeters^d, and Nicholas H. Oberlies^{b,*}

^a Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid 22110, Jordan

^b Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, North Carolina 27402, United States

^c Department of Chemistry, Wake Forest University, Winston-Salem, North Carolina 27109, United States

^d Department of Chemistry, University of Alabama in Huntsville, Huntsville, Alabama 35899, United States

ARTICLE INFO

Article history:

Received

Received in revised form

Accepted

Available online

Keywords:

Freshwater fungi

α -Pyrone

Tetrahydroxanthones

Cyclodepsipeptides

Bacterial peptidyl-tRNA hydrolase

ABSTRACT

Eighteen (**1–18**) and seven (**1, 4, 6–8, 17** and **18**) compounds were isolated from organic extracts of axenic cultures of two freshwater fungi *Clohesyomyces* sp. and *Clohesyomyces aquaticus* (Dothideomycetes, Ascomycota), respectively. Compounds **1–12** belong to the α -pyrone class of natural products, compounds **13** and **14** were tetrahydroxanthones, compounds **15** and **16** were hexahydroxanthones, while compounds **17** and **18** were cyclodepsipeptides. The structures were elucidated using a set of spectroscopic and spectrometric techniques. The absolute configurations of compounds **2, 3, 6**, and **7** were assigned via a modified Mosher's ester method using ¹H NMR data. The relative configurations of compounds **14–16** were determined through NOE data. Compounds **1, 2, 6, 8, 13, 14**, and **15** were found to inhibit the essential enzyme bacterial peptidyl-tRNA hydrolase (Pth1), with (**13**; secalonic acid A) being the most potent. Compounds **1** and **4–18** were also evaluated for antimicrobial activity against an array of bacteria and fungi but were found to be inactive.

2009 Elsevier Ltd. All rights reserved.

1. Introduction

In pursuit of structurally unique and bioactive secondary metabolites from distinct ecological habitats, our group has been interested in fungi, particularly freshwater fungi^{1–5} that inhabit submerged woody and herbaceous substrates in streams and lakes.⁶ Freshwater fungi represent a potentially rich, although poorly studied, source of new mycological and chemical diversity.^{7,8}

While working in the Lake Brandt watershed of Greensboro, NC, two related freshwater fungal species, belonging to the genus *Clohesyomyces*, were isolated from submerged wood and accessioned as G100 and G102. Fungal strain G102 biosynthesized 18 compounds belonging to three distinct classes of natural products. Twelve were members of the α -pyrone class of secondary metabolites (**1–12**), of which five were known [phomopsinone A (**1**), phomopsinone B (**2**), phomopsinone C (**3**), pyrenocine M (**4**), and pyrenocine K (**5**)] and seven were new [6-hydroxy-7-*epi*-phomopsinone A (**6**), 5-deoxy-7-hydroxypyrenocine M (**7**), pyrenocine P (**8**), 7-hydroxypyrenocine M (**9**), pyrenocine Q (**10**), pyrenocine R (**11**),

5-hydroxyphomopsinone A (**12**)]. Alternatively, four metabolites were tetra/hexahydroxanthones (**13–16**), of which one was the known homodimeric tetrahydroxanthone, secalonic acid A (**13**), another was a new natural monomeric tetrahydroxanthone [8-hydroxyblennolide H (**14**)], and two were new monomeric hexahydroxanthones [*cis*-dihydro-8-hydroxyblennolide H (**15**) and *trans*-dihydro-8-hydroxyblennolide H (**16**)]. Finally, also isolated were the two known cyclodepsipeptides [Sch 378161 (**17**) and Sch 217048 (**18**)]. From the perspective of chemical mycology, it was interesting that of these 18 metabolites, the related strain G100 biosynthesized seven overlapping compounds, being **1, 4, 6–8, 17** and **18**, which belong to the α -pyrone and cyclodepsipeptide classes of compounds, while the tetra/hexahydroxanthones were biosynthesized only by the G102 strain. Since these related fungi were isolated from the same freshwater habitat, and because they biosynthesized an overlapping series of compounds, the chemical, biological, and mycological data were combined into a single communication.

α -Pyrone have been reported to have varying potency in assays for antifungal, antibacterial, and antialgal activity.^{9,10} Tetrahydroxanthones are an important class of mycotoxins with

* Corresponding author. Tel.: +1-336-334-5474; fax: +1-336-334-5402; e-mail: n_oberli@uncg.edu

diverse biological activities, including antitumor, antibacterial, and anti-HIV.^{11–17} The isolated cyclodepsipeptides in this study were reported as selective and competitive human tachykinin receptor (NK₂) antagonists.¹⁸ Given this diversity of biological activities, compounds **1–18** were evaluated against a panel of bacteria and fungi. While *in vivo* activity was without prospect, *in vitro* inhibitory activity against the essential enzyme bacterial peptidyl-tRNA hydrolase (Pth1) was observed. Bacterial Pth1 represents a promising new target for antibiotic development¹⁹ for which little is known about small molecule inhibition.²⁰ Based on this initial screening set, secondary metabolites from fungal cultures could serve as a source of Pth1 inhibitors, providing avenues to better understand inhibitor binding and inhibitor bound states.

2. Results and Discussion

The freshwater fungal strains, coded G100 and G102, were ITS sequenced²¹ and identified as *Clohesyomyces aquaticus* (Dothideomycetes, Ascomycota) and *Clohesyomyces* sp., respectively. Large-scale solid-phase cultures of G100 and G102 were extracted and partitioned with organic solvents, as described previously.^{3,5} The organic extracts were then purified separately using normal-phase flash chromatography to generate five fractions each, which were then subjected to further purifications using preparative and semipreparative HPLC to yield seven (**1**, **4**, **6–8**, **17** and **18**) and 18 compounds (**1–18**) from G100 and G102, respectively; all compounds were verified to have >95% purity as measured by UPLC using an evaporative light scattering detector (ELSD) (Figure S1, Supporting Information). The biosynthetic potential of these two fungal cultures was broad, as compounds **1–12** belonged to the α -pyrone class of natural products, compounds **13–16** were tetra/hexahydroxanthones, while compounds **17** and **18** were cyclodepsipeptides.

2.1. α -Pyrone derivatives

The NMR and HRESIMS, along with CD and optical rotation data, where appropriate, identified compounds **1** [6.1 mg (G100); 27.8 mg (G102)]; **2** [0.8 mg (G102)], **3** [0.7 mg (G102)], **4** [0.8 mg (G100); 1.7 mg (G102)], and **5** [0.5 mg (G102)] as the known metabolites phomopsinone A,¹⁰ phomopsinone B,¹⁰ phomopsinone C,¹⁰ pyrenocine M,⁹ and pyrenocine K,⁹ respectively (Figures 1 and S2–S6, Table S1). Compounds **1** and **4** were first isolated from two different strains of an endophytic *Phomopsis* sp., which were isolated from two different plant hosts.^{9,10} The specific rotation data of **1** was not reported previously and hence provided herein. Epimeric **2** and **3** were first isolated from an endophytic *Phomopsis* sp., which was isolated from *Santolina chamaecyparissus*.¹⁰ The absolute configuration of **2** was determined by CD spectroscopy and TDDFT calculations.¹⁰ However, due to paucity and insufficient purity of **3**, the researchers were unable to collect CD data to assign the absolute configuration. Hence, they relied on biogenetic considerations and the fact that **2** and **3** were epimers at C-10 to propose the absolute configuration for **3**. No specific rotation data were reported for compounds **2** and **3**. Herein, the absolute configurations of **2** and **3** were confirmed via a modified Mosher's ester method (Figure 2),²² the specific rotation data for **2** and **3** and the CD data for **3** were provided as well (Figure 3). Finally, compound **5** was identified as the known pyrenocine K, which was first isolated from a *Phomopsis* sp., an endophyte of *Cistus salviifolius*.⁹ There were some minor differences between the reported and the collected NMR data for **5** (Table S2); however the 2D-NMR and specific rotation data confirmed the structure of **5** as the known pyrenocine K.⁹ The NMR data are provided to update the literature (Table 1). CD data have not

been reported for compound **5** and hence they are provided (Figure 3) to confirm the absolute configuration as 7*R*. In short, the characterization data for five of the previously reported α -pyrones have been refined.

Figure 1. Structures of metabolites isolated from fungal cultures G100 and G102 (**1–18**).

Figure 2. $\Delta\delta_{\text{H}}$ values [$\Delta\delta$ (in ppm) = $\delta_{\text{S}} - \delta_{\text{R}}$] obtained for (S)- and (R)-MTPA esters A) **2a** and **2b**, respectively of phomopsinone B (**2**), B) **3a** and **3b**, respectively of phomopsinone C (**3**), C) **6a** and **6b**, respectively of 6-hydroxy-7-*epi*-phomopsinone A (**6**), and D) **7a** and **7b**, respectively of 5-deoxy-7-hydroxypyrenocine M (**7**) in pyridine-*d*₅.

Figure 3. ECD spectra of A) phomopsinone C (**3**) [0.58 mM] in CH_3CN ; B) pyrenocine K (**5**) [0.25 mM] in CH_3OH ; C) 6-hydroxy-7-*epi*-phomopsinone A (**6**) [0.12 mM] in CH_3CN ; D) pyrenocine P (**8**) [0.28 mM] in CH_3OH ; E) 5-hydroxyphomopsinone A (**12**) [0.13 mM] in CH_3CN , and F) *cis*-dihydro-8-hydroxyblennolide H (**15**) [0.19 mM] and *trans*-dihydro-8-hydroxyblennolide H (**16**) [0.22 mM] in CHCl_3 , cell length 2 cm.

Table 1

^1H NMR Data (700 MHz) for **5** and (500 MHz) for **6**, **8**, and **12** in CDCl_3 .

position	5	6	8	12
3	5.43, s	5.48, s	5.45, s	5.44, s
5	4.33, dt (14.9, 2.9)	4.38, dd (15.5, 2.3)	4.38, dtd (14.9, 2.9, 0.6)	5.87, d (4.0)
	4.52, dd (14.9, 1.2)	4.51, dd (15.5, 1.7)	4.63, ddd (14.9, 1.7, 0.6)	
6	2.47, m	4.21, dddd (7.9, 2.9, 2.3, 1.7)	2.38, dt (17.2, 2.9)	2.44, m
7	2.52, m		2.62, m	
	4.12, m	3.48, ddd (8.7, 7.9, 2.9)	3.79, m	4.31, m
8	2.60, dd (17.0, 4.7)	1.55, m	3.66, m	1.57, m
	2.85, dd (17.0, 7.3)	1.82, m	3.79, m	1.64, m
9		1.43, m	3.81, s	1.42, m
		1.58, m		1.49, m
10	2.20, s	0.95, t (7.5)		0.95, t (7.5)
11	3.79, s	3.82, s		3.84, s
5-OH				2.81, d (4.0)
6-OH		2.68, d (2.9)		
8-OH			2.04, t (6.3)	

$^a\delta$ in ppm, mult (J in Hz).

Compound **6** [2.0 mg (G100); 1.2 mg (G102)] was obtained as a colorless solid with a molecular formula of $\text{C}_{12}\text{H}_{16}\text{O}_5$ as determined by HRESIMS along with ^1H , ^{13}C , and edited-HSQC NMR data (Tables 1 and 2, Figure S7), establishing an index of hydrogen deficiency of 5. The NMR data suggested that **6** was an α -pyrone analogue of **1**. A key difference was replacement of the allylic methylene moiety ($\delta_{\text{H}}/\delta_{\text{C}}$ 2.40/32.5, dd, $J = 6.4$, 1.8 for $\text{H}_2\text{-6/C-6}$) in **1** by an oxymethine in **6** ($\delta_{\text{H}}/\delta_{\text{C}}$ 4.21/66.6, dddd, $J = 7.9$, 2.9, 2.3, 1.7 for H-6/C-6). These data, along with a 16 amu difference in the HRMS data of compounds **1** and **6**, indicated hydroxylation at the C-6 position in **6**. The coupling constant ($J_{\text{H-6/H-7}} = 7.9$ Hz) implied a pseudoaxial/pseudoequatorial *cis* orientation in **6** (Table 1, Figure S7). NOESY correlations observed between H-6 and H-7 indicated that these two protons were on the same face (Figure 4). An HMBC correlation was observed from 11- OCH_3 to C-4, indicating the connectivity of the methoxy group (Figure 5). HMBC correlations from H-3 to C-4, C-4a, and C-2, from $\text{H}_2\text{-5}$ to C-6a and C-7, from H-6 to C-4a and

C-8, from H-7 to C-9, and from $\text{H}_3\text{-10}$ to C-8 were observed (Figure 5). The trivial name 6-hydroxy-7-*epi*-phomopsinone A was ascribed to compound **6**. The absolute configuration of **6** was assigned via a modified Mosher's ester method,²² establishing the configurations as 6*S* and 7*R* (Figure 2C), which were opposite to that reported for the known phomopsinone D.¹⁰ Moreover, 6-hydroxy-7-*epi*-phomopsinone A (**6**) and phomopsinone D showed opposite CD spectra (Table S1, Figure 3). The specific rotation data for phomopsinone D have not been reported in the literature previously.

Table 2

^{13}C NMR Data for **5** and **10** (175 MHz) and for **6-9**, **11-12** (125 MHz) in CDCl_3 .

position	5	6	7	8	9	10	11	12
2	164.2	163.8	164.6	164.1	164.3	165.4	164.1	163.8
3	88.3	88.9	88.1	88.2	88.6	102.0	89.3	88.4
4	168.5	168.2	171.0	168.4	170.1	165.9	170.8	168.6
4a	107.6	109.1	108.9	107.6	113.7	92.9	108.6	108.9
5	61.9	61.4	9.71	61.9	54.2	8.8	9.1	87.4
6	32.3	66.6	38.9	28.3	38.5	71.4	59.3	32.6
6a	155.9	155.9	158.2	155.9	161.9	165.0	157.0	160.1
7	70.2	78.8	69.8	74.4	69.2	35.5	56.5	66.2
8	48.9	34.3	39.5	64.9	40.0	27.4		37.2
9	205.7	18.7	18.9	56.2	18.9	22.6		18.6
10	31.2	14.1	14.1		14.1	14.2		14.05
11	56.3	56.4	56.2		56.6	56.6		56.5

$^a\delta$ in ppm.

Compound **7** [1.7 mg (G100); 0.7 mg (G102)] was obtained as a colorless solid with a molecular formula of $\text{C}_{12}\text{H}_{18}\text{O}_4$ as determined by HRESIMS along with ^1H , ^{13}C , and edited-HSQC NMR data (Tables 2 and 3, Figure S8), indicating an index of hydrogen deficiency of 4. These NMR data suggested another α -pyrone analogue with structural similarities to **1**. However, a critical difference was replacement of the allylic oxymethylene moiety ($\delta_{\text{H}}/\delta_{\text{C}}$ 4.28/4.53/61.6, dt, $J = 14.7$, 2.8 and d, $J = 14.7$ for H-5a/H-5b/C-5) in **1** by a singlet methyl group in **7** ($\delta_{\text{H}}/\delta_{\text{C}}$ 1.90/9.71). These data, along with a 2 amu difference in the HRMS data of compounds **1** and **7** and a decrease in hydrogen deficiency, indicated a cleavage of the 3,6-dihydropyran ring in **7** relative to **1**. HMBC correlations were observed from $\text{H}_3\text{-5}$ to C-4 and C-6a, from $\text{H}_2\text{-6}$ to C-4a, C-6a, and C-8, from $\text{H}_3\text{-10}$ to C-8, from 11- OCH_3 to C-4, and from H-3 to C-4 and C-4a (Figure 5). The trivial name 5-deoxy-7-hydroxypyrenocine M was ascribed to compound **7**, and the absolute configuration was assigned as 7*R* via a modified Mosher's ester method²² (Figure 2).

Figure 4. Key NOESY correlations of **6**, **12**, and **14-16**.

Figure 5. Key COSY and HMBC correlations of compounds **6–12** and **14–16**.

Compound **8** [2.5 mg (G100); 2.4 mg (G102)] was obtained as a colorless solid with a molecular formula of $C_{10}H_{12}O_5$ as determined by HRESIMS along with 1H , ^{13}C , and edited-HSQC NMR data (Tables 1 and 2, Figure S9), establishing an index of hydrogen deficiency of 5. These NMR data suggested **8** as an α -pyrone derivative with structural similarity to **1**. Key differences were observed in the aliphatic side chain, where the terminal ethyl moiety in **1** was replaced by a hydroxy group in **8**. These considerations, along with the COSY and HMBC data, established the structure of **8** (Figure 5), which was confirmed via single crystal X-ray diffraction (Figure 6); ECD data suggested the absolute configuration as **7S**. The trivial name pyrenocine P was ascribed to compound **8**.

Figure 6. X-ray crystallographic structure of **8**.

Compound **9** [6.9 mg (G102)], which was also obtained as a colorless solid, was found to have a molecular formula of $C_{12}H_{18}O_5$ as determined by HRESIMS along with 1H , ^{13}C , and edited-HSQC NMR data (Tables 2 and 3, Figure S10A), establishing an index of hydrogen deficiency of 4. The structure of **9** was determined as an α -pyrone derivative by comparison of its NMR and HRMS data with those of **4**. A key difference was replacement of the methylene moiety (δ_H/δ_C 1.68/27.9, m, H₂-7/C-7) in **4** by an oxymethine in **9** (δ_H/δ_C 4.01/69.2, m, for H-7/C-7). These data, along with a 16 amu difference in the HRMS data of compounds **4** and **9**, indicated hydroxylation at C-7 in **9**. HMBC correlations from H-3 to C-4, C-4a, and C-2, from H₂-5

to C-6a and C-4, from H₂-6 to C-4a and C-8, from H₂-9 to C-7, and from H₃-10 to C-8 were observed (Figure 5). The trivial name 7-hydroxypyrenocine M was ascribed to compound **9**. In an attempt to determine the absolute configuration of **9** via a modified Mosher's ester method,²² the compound was found to be racemic, suggesting that it may be an artifact of the purification processes (Figure S10B).

Table 3

1H NMR Data (500 MHz) for **7** and **9–11** in $CDCl_3$ ^a

position	7	9	10	11
3	5.46, s	5.47, s		5.50, s
4a			6.34, s	
5	1.90, s	4.30, d (12.6)	1.90, s	1.93, s
		4.51, d (12.6)		
6	2.62, dd (14.3, 4.0)	2.63, dd (14.3, 2.9)	4.42, m	4.46, d (6.3)
	2.69, dd (14.3, 8.6)	2.75, dd (14.3, 9.7)		
7	4.07, m	4.01, m	1.70, m	3.83, s
			1.87, m	
8	1.50, m	1.53, m	1.36, m	
			1.42, m	
9	1.38, m	1.39, m	1.34, m	
	1.50, m	1.46, m		
10	0.93, t (6.9)	0.94, t (7.5)	0.89, t (6.9)	
11	3.81, s	3.85, s	3.90, s	
5-OH		3.29, br.		
6-OH			2.02, d (5.7)	2.22, t (6.3)
7-OH		2.46, br.		

^a δ in ppm, mult (J in Hz).

Compound **10** [0.7 mg (G102)] was obtained as a colorless solid with a molecular formula of $C_{12}H_{18}O_4$ as determined by HRESIMS and analysis of 1H , ^{13}C , and edited-HSQC NMR data (Tables 2 and 3, Figure S11). Inspection of the NMR data suggested compound **10** as an α -pyrone analogue with structural similarity to **7**, both sharing the same molecular formula. However, the allylic methyl singlet (δ_H/δ_C 1.90/9.71, s, for H₃-5/C-5) in **7** was replaced by an olefinic proton (δ_H/δ_C 6.34/92.9, s, for H-4a/C-4a) in **10**, and the olefinic proton (δ_H/δ_C 5.46/88.1, s, for H-3/C-3) in **7** was replaced by an allylic methyl singlet (δ_H/δ_C 1.90/8.8, s, for H₃-5/C-5) in **10**. These data indicated that the olefinic proton and the methyl group switched positions, which was confirmed by observed HMBC correlations from H-4a to C-6 and C-6a (Figure 5). Another key difference included a 1,2-hydroxy shift in **10** relative to **7**. The allylic methylene moiety ($\delta_H/\delta_H/\delta_C$ 2.62/2.69/38.9, dd, $J = 14.3, 4.0$ and $J = 14.3, 8.6$, for H-6a/H-6b/C-6, respectively) in **7** was replaced by an allylic carbinol carbon (δ_H/δ_C 4.42/71.4, m, for H-6/C-6), while the carbinol carbon (δ_H/δ_C 4.07/69.8, m, for H-7/C-7) in **7** was replaced by a methylene moiety ($\delta_H/\delta_H/\delta_C$ 1.70/1.87/35.5, m, for H-7a/H-7b/C-7). This shift was confirmed by the observed HMBC correlations from 6-OH to C-6a and C-7, from H₂-9 to C-7, and from H-6 to C-8 (Figure 5). Further examination of the NMR data, including COSY and HMBC spectra (Figure 5), yielded the structure of **10**, which was ascribed the trivial name pyrenocine Q. As for **9**, an attempt to determine the absolute configuration of **10** via a modified Mosher's ester method²² revealed a racemic mixture of the *R* and *S* enantiomers.

Compound **11** [0.5 mg (G102)] was isolated as a colorless solid with a molecular formula of $C_8H_{10}O_4$ as revealed by analysis of the HRESIMS, 1H NMR, ^{13}C NMR and edited-HSQC NMR data (Tables 2 and 3, Figure S12), indicating an index of hydrogen deficiency of four. Inspection of the NMR data indicated **11** was a relatively simple α -pyrone derivative, but with differences in the aliphatic side chain and the 3,6-dihydropyran ring. In addition to key NMR resonances for the α -pyrone ring, NMR data indicated the presence of a methoxy group, an allylic methyl singlet and an allylic hydroxymethyl group (Tables 2 and 3, Figure S12). These data suggested a trisubstituted α -pyrone derivative. HMBC correlations from H₂-6 to C-4a, from H₃-5 to C-6a, from H₃-7 to C-4, and from H-3 to C-4a confirmed the substituents' positions (Figure 5). Further examination of the NMR data yielded the structure of **11**, which was ascribed the trivial name pyrenocine R.

Compound **12** [0.9 mg (G102)], which was isolated as a colorless solid, had a molecular formula of $C_{12}H_{16}O_5$ as deduced from HRESIMS along with 1H NMR, ^{13}C NMR, and edited-HSQC NMR data (Tables 1 and 3, Figure S13), indicating an index of hydrogen deficiency of 5. Analysis of the NMR data indicated **12** as an α -pyrone derivative with structural similarity to **1**. A key difference was replacement of the allylic oxymethylene moiety (δ_H/δ_C 4.30/4.57/61.7, dt, $J = 14.9, 2.9$; d, $J = 14.9$, for H-5a/H-5b/C-5) in **1** by a dioxymethine group in **12** (δ_H/δ_C 5.87/87.4, d, $J = 4.0$, for H-5/C-5). These data, along with a 16 amu difference in the HRMS data of compounds **1** and **6**, indicated hydroxylation at the C-5 position in **12**, which was further confirmed by observation of an NMR signal for the -OH group (δ_H 2.81, d, $J = 4.0$) that showed diagnostic HMBC correlations with C-5 and C-4a (Table 3, Figure 5). Further analysis of the NMR spectra, including COSY and HMBC data, yielded the structure of **12**, which was given the trivial name 5-hydroxyphomopsinone A. The similarity of the ECD data of **12** (Figure 3) with that of **1** (Figure S2), along with the observed NOESY correlation from 5-OH to H-7, were used to establish the absolute configuration as 5*R* and 7*S* (Figure 4).

2.2. Monomeric/dimeric tetra/hexahydroxanthones

Compounds **13**–**16** were identified as a homodimeric tetrahydroxanthone (**13**), a monomeric tetrahydroxanthone (**14**), and a pair of monomeric hexahydroxanthones (**15**–**16**). Analysis of HRMS, NMR, and ECD data identified **13** [7.4 mg (G102)] as the known secalonic acid A (Figures S2 and S14).^{17,23} Structurally related monomeric/dimeric tetrahydroxanthones, including **13**, were isolated previously in our lab from an organic extract of the filamentous fungal culture *Setophoma terrestris*.¹⁷

Compound **14** [2.3 mg (G102)] was obtained as a colorless solid with a molecular formula of $C_{16}H_{16}O_6$ deduced by HRESIMS and analysis of 1H NMR, ^{13}C NMR and edited-HSQC NMR data (Table 4, Figure S15), indicating an index of hydrogen deficiency of 9. Analysis of the HRMS and NMR data suggested **14** as a tetrahydroxanthone derivative with structural similarity to the monomeric units of **13**. However, a key difference was replacement of the C-2 quaternary carbon in **13** (δ_C 118.3) by an aromatic methine in **14** (δ_H/δ_C 6.52/110.9, d, $J = 8.0$). COSY data identified two spin systems as H-2/H-3/H-4 and H-5/H-6/H-7 (Figure 5). Further examination of the NMR spectra, including HMBC data (Figure 5), yielded the gross structure of **14**, which was identical to the monomeric units of **13**. A NOESY correlation was observed between 11-CH₃ and H-5, indicating that those protons were on the same side of the molecule (Figure

4). A proton doublet corresponding to H-5 (δ_H 3.90, d, $J = 10.9$ Hz) implied a pseudodiaxial *trans* orientation of H-5/H-6, as observed in the monomeric units of **13**.¹⁷ These data along with biogenetic considerations established the relative configuration of **14** to be (5*S*,6*R*,10*aS*), the same as that of the monomeric units of **13**. Due to the structural similarity of **14** with blennolide H, a tetrahydroxanthone derivative that has been recently isolated in our lab,¹⁷ compound **14** was ascribed the trivial name 8-hydroxyblennolide H. Moreover, compound **14** and blennolide H showed similar ECD spectra (Figure S2), confirming the assignment of absolute configuration. Compound **14** was reported recently by synthesis and was described using the name (–)-blennolide B.²⁴ (–)-Blennolide B is an enantiomer of the natural blennolide B (which is dextro rotatory).²⁵ Due to this confusion with the trivial name, and because this is the first isolation of the natural product, we believe that 10-hydroxyblennolide H is a more appropriate and understandable name.

Diastereoisomers **15** [0.6 mg (G102)] and **16** [1.0 mg (G102)] were obtained as colorless oils, both having molecular formulae of $C_{16}H_{18}O_7$, as determined by HRESIMS and analysis of 1H NMR, ^{13}C NMR and edited-HSQC NMR data (Table 4, Figures S16 and S17), establishing an index of hydrogen deficiency of 8. Compounds **15** and **16** showed similar 1H and ^{13}C NMR spectra (Table 4, Figures S16 and S17) with small differences in the chemical shift values correlating to ring C (δ_H/δ_C 3.52/78.1, d, $J = 10.9$, for H-5/C-5 in **15** and 3.76/79.2, dd, $J = 10.9, 3.4$, for H-5/C-5 in **16** and δ_H/δ_C 4.37/67.9, d, $J = 4.0$, for H-8/C-8 in **15** and 4.82/66.0, m, for H-8/C-8 in **16**). Interestingly, compounds **15** and **16** displayed opposite signs for optical rotation data; while compound **15** was levorotatory (-72.7°), compound **16** was dextrorotatory ($+38.2^\circ$). Moreover, the ECD spectra of **15** and **16** were mirror images (Figure 3). The HRMS and NMR data indicated **15** and **16** as dihydro analogues of **14**, as evidenced by both a 2 amu difference in the HRMS data and replacement of the C-8 (δ_C 177.7) and C-8a (δ_C 101.8) quaternary olefinic carbons in **14** by oxymethines and methines, respectively in **15** and **16**, (δ_H/δ_C 4.37/67.9, d, $J = 4.0$, for H-8/C-8 in **15** and 4.82/66.0, m, for H-8/C-8 in **16**) and (δ_H/δ_C 3.12/52.4, d, $J = 4.0$, for H-8a/C-8a in **15** and 2.94/56.2, d, $J = 9.2$, for H-8a/C-8a in **16**). COSY data identified the following spin systems H-5/H-6/(CH₃-11)/H₂-7/H-8/H-8a and H-2/H-3/H-4 in **15** and 5-OH/H-5/H-6/(CH₃-11)/H₂-7/H-8/(8-OH)/H-8a and H-2/H-3/H-4 in **16** (Figure 5). These data, along with the HMBC correlations (Figure 5), established the gross structures of **15** and **16**. The relative configuration of ring C at the C-5, C-6, and C-10a

Table 4
 1H (500 MHz) and ^{13}C NMR (125 MHz) for **14** and **16**, and (100 MHz) for **15**^a

Position	14		15		16	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	162.2		162.1		162.0	
2	110.9	6.52, d (8.0)	110.5	6.53, d (8.0)	110.7	6.53, d (8.6)
3	138.3	7.35, dd (8.6, 8.0)	139.0	7.40, t (8.0)	139.2	7.40, dd (8.6, 8.0)
4	108.1	6.55, d (8.6)	107.7	6.59, d (8.0)	107.9	6.54, d (8.0)
4a	159.0		160.3		159.7	
5	77.1	3.90, d (10.9)	78.1	3.52, d (10.9)	79.2	3.76, dd (10.9, 3.4)
6	29.4	2.40, m	28.5	2.26, m	31.7	1.84, m
7	36.4	2.29, dd (19.5, 10.9)	39.7	1.53, ddd (14.3, 12.6, 2.9)	39.0	1.27, dd (13.2, 11.5)
		2.72, dd (19.5, 6.3)		2.02, dt (14.3, 3.4)		2.13, ddd (13.2, 5.2, 4.0)
8	177.7		67.9	4.37, d (4.0)	66.0	4.82, m
8a	101.8		52.4	3.12, d (4.0)	56.2	2.94, d (9.2)
9	187.2		196.9		199.0	
9a	107.2		109.8		107.7	
10a	84.9		85.0		87.8	
11	18.2	1.15, d (6.3)	17.9	1.15, d (6.9)	18.3	1.11, d (6.3)
12	170.5		171.1		169.3	
13	53.4	3.68, s	53.6	3.67, s	53.3	3.62, s
1-OH		11.23, s		11.58, s		11.12, s
5-OH		2.77, br.				2.67, d (3.4)
8-OH						3.64, d (1.7)

^a δ in ppm, mult (J in Hz).

stereogenic centers in compounds **15** and **16** was similar based on NOESY correlations between H₃-11 and H-5 and coupling constant values of 10.9 in compounds **15** and **16**, implying a pseudodiaxial *trans* orientation of H-5/H-6 (Figure 4, Table 4), and the configurations were presumed similar to those of **13** due to biogenetic origins.¹⁷ The 3D arrangements of **15** and **16** were different, as observed via NOESY data, particularly between H-8a to H-5 and H-8 in **15** and between H-8 to H-6 and H-8a to H-5 in **16** (Figure 4). Putting these data together established compounds **15** and **16** as epimers at C-8 with the relative configuration of **15** as (5*S*,6*R*,8*R*,8a*R*,10*aS*) and of **16** as (5*S*,6*R*,8*S*,8a*R*,10*aS*). The trivial names *cis*-dihydro-8-hydroxyblennolide H and *trans*-dihydro-8-hydroxyblennolide H were ascribed to compounds **15** and **16**, respectively.

2.3. Cyclodepsipeptides

In addition to compounds **1–16**, two known cyclodepsipeptides, Sch 378161 (**17**) [4.6 mg (G100); 1.4 mg (G102)] and Sch 217048 (**18**) [10.7 mg (G100); 1.6 mg (G102)], were isolated and identified by means of NMR and HRMS and MS/MS data (Figure S18), all of which compared favorably to the literature.^{18,26}

2.4. Antimicrobial Activity

Compounds **1** and **4–18** were tested for antimicrobial activity against an array of bacteria and fungi (*Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium smegmatis*, *Candida albicans*, and *Aspergillus niger*), but were found inactive. Compounds **1**, **2**, **6**, **8**, **13**, **14**, and **15** showed varying degrees of *in vitro* inhibition against bacterial Pth1 from several species. All but the most potent inhibitor lead to a decreased solubility of the enzyme under the conditions tested. A cloudiness in the reaction appeared upon addition of these compounds and was determined to be insoluble Pth1 from commassie gel of the pelleted material after centrifugation. The degree of Pth1 aggregation was somewhat correlated to the amount of compound added, but also was effected by speed of compound addition, concentration of compound addition, temperature of the reaction, and was partially reversible over the course of the assay. Thus, while these compounds do appear to interact with Pth1, no meaningful quantitation of inhibition was possible. The most potent inhibitor was **13**, demonstrating an IC_{50} of 1.4 mM against *Salmonella typhimurium* Pth1 (Figure 7). From chemical shift perturbation mapping, **13** was confirmed to bind in the active site (Figure 8). Helping to explain the low affinity, **13** also bound to at least one other site on Pth1, in part the same secondary site which did not affect enzyme activity for a previously reported synthetic inhibitor.²⁰ While not promising for clinical application, these findings represent the first reported small molecule natural product inhibitor of bacterial Pth1, which to date has been limited to crude natural product extracts.^{27,28} Therefore these compounds provide tools to expand our understanding of Pth1 inhibition. With the tremendous structural biology knowledge of apo Pth1, having an inhibitor enables an array of follow-up structure-function studies, far out weighing the lack of potency. Of particular interest is high resolution structural characterization of an inhibitor bound complex, providing understanding of small molecule Pth1 inhibition, for which there is no substrate or inhibitor bound complex known to date. Also, having inhibitors with known structures provides a means to help bridge the gap between computational docking and empirical inhibition.²⁹

Figure 7. Concentration-% inhibition curve of compound **13** against bacterial peptidyl-tRNA hydrolase (Pth1).

Figure 8. 1H - ^{15}N Chemical shift perturbations caused by **13** (secalonic acid A) binding to *E. coli* Pth1. At top, the ribbon diagram of *E. coli* Pth1 indicates residues with chemical shift perturbations between one third and one peak width in cyan and those greater than the peak width in blue. Catalytically essential His20 is red, with the side chain in the active site cleft shown. Below, the corresponding surface representations of Pth1 are colored as in part A. The cluster of perturbed residues (black arrow) indicate an interaction distinct from the Pth1 active site.

3. Conclusions

From two fungal cultures that were isolated from submerged wood in a lake, 18 compounds (**1–18**) belonging to three structural classes, α -pyrone, tetra/hexahydroxanthones, and cyclodepsipeptides, were isolated and characterized. The studied fungi, G100 and G102, shared the ability to biosynthesize two classes of natural products, α -pyrone and cyclodepsipeptides. On the other hand, G102 has the additional biosynthetic capabilities to generate tetrahydroxanthone derivatives. The absolute configuration of the known (**2** and **3**) and the new (**6** and **7**) α -pyrone derivatives were assigned via a modified Mosher's ester method. The relative configurations of the tetra/hexahydroxanthones (**14–16**) were determined through NOE data. Via testing these compounds against a new assay for inhibition of bacterial Pth1,³⁰ the first natural product inhibitor (**13**) for this potential antibiotic target was identified. While target based screening for antibacterials has many well documented challenges,³¹ identification of Pth1 inhibitors opens the possibility for characterization of an inhibitor bound complex, which may, in turn, lead to more meaningful use of computational aspects aimed at inhibitor design/development. Interestingly, the dimeric tetrahydroxanthone class of compounds, such as **13**, seems to be attracting interest of the synthetic community.²⁴ The current study is another demonstration⁴ that understudied biodiversity, regardless of its proximity to urban vs rural vs extreme locations, often reveals new chemical diversity.

4. Experimental

4.1. General experimental procedures

Optical rotations, UV data, and ECD spectra were obtained using a Rudolph Research Autopol III polarimeter (Rudolph Research Analytical), a Varian Cary 100 Bio UV-vis spectrophotometer (Varian Inc.), and an Olis DSM 17 ECD

spectrophotometer (Olis, Inc.). NMR data were collected using either a JEOL ECA-500 NMR spectrometer operating at 500 MHz for ^1H and 125 MHz for ^{13}C , or a JEOL ECS-400 NMR spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C and equipped with a high sensitivity JEOL Royal probe and a 24-slot autosampler (both from JEOL Ltd.), or an Agilent 700 MHz NMR spectrometer (Agilent Technologies), equipped with a cryoprobe, operating at 700 MHz for ^1H and 175 MHz for ^{13}C . Residual solvent signals were utilized for referencing. HRMS utilized a Thermo LTQ Orbitrap XL mass spectrometer equipped with an electrospray ionization source (Thermo Fisher Scientific). A Waters Acquity UPLC system (Waters Corp.) utilizing a Waters BEH C_{18} column (1.7 μm ; 50 $\times$ 2.1 mm) was used to evaluate the purity of the isolated compounds with data collected and analyzed using Empower 3 software. Phenomenex Gemini-NX C_{18} analytical (5 μm ; 250 $\times$ 4.6 mm), preparative (5 μm ; 250 $\times$ 21.2 mm), and semipreparative (5 μm ; 250 $\times$ 10.0 mm) columns (all from Phenomenex) were used on a Varian Prostar HPLC system equipped with ProStar 210 pumps and a Prostar 335 photodiode array detector (PDA), with data collected and analyzed using Galaxie Chromatography Workstation software (version 1.9.3.2, Varian Inc.). Flash chromatography was performed on a Teledyne ISCO CombiFlash Rf 200 using Silica Gold columns (both from Teledyne Isco) and monitored by UV and evaporative light-scattering detectors. Single crystal X-ray diffraction data were collected using a Bruker APEX CCD diffractometer (Mo $\text{K}\alpha$ radiation, graphite monochromator). The crystallographic images were generated using CrystalMaker (CrystalMaker Software). All other reagents and solvents were obtained from Fisher Scientific and were used without further purification.

4.2. Fungal strains isolation and identification

Fungal strains G100 and G102 were isolated from conidia that were found on samples of submerged wood collected from Lake Brandt (36°10'01.0"N 79°50'18.0"W) in Greensboro, North Carolina, USA in October 2011. Collecting methods and culturing conditions utilized protocols outlined previously.³²⁻³⁴ Identifications were based on observation of micromorphological characters using an Olympus microscope (BX53) equipped with an Olympus DP25 camera. Molecular sequence data were obtained from internal transcribed spacer regions 1 & 2 and 5.8S nrDNA (ITS).²¹ DNA extraction, PCR amplification, and sequencing were performed using previously described methods.^{1,2,35} To obtain identification via sequence comparisons, the ITS region was BLAST searched against both GenBank and the UNITE databases.³⁶ Results from BLAST search suggested sequence similarities between G100 and G102 to certain taxa within the order Pleosporales, Dothideomycetes, Ascomycota. G100 showed similarity (94%) to *Clohesyomyces aquaticus*,^{37,38} whereas, G102 showed similarity (98%) to *Clohesyomyces* sp. (MAFF 239218; GenBank: JQ435791). Interestingly, G100 was also morphologically identical to *Clohesyomyces aquaticus* (Figure S20). The sequences with high homology were downloaded and analyzed using Maximum Likelihood³⁹ to phylogenetically evaluate the evolutionary affinities of the two isolates (G100, and G102). Based on the results of morphology, BLAST search, and phylogenetic analysis of the ITS region, strain G100 was identified as *Clohesyomyces aquaticus*, while strain G102 was identified as having phylogenetic affinities to *Clohesyomyces* sp. (MAFF 239218; GenBank: JQ435791) (Figure S19). The ITS sequences for both strains were deposited in the GenBank (G100: KM589855; G102: KU926856). Voucher cultures of strain G100 and G102 were maintained in the Department of Chemistry and Biochemistry culture collection at the University of North Carolina at Greensboro.

4.3. Fermentation, extraction and isolation

Extraction and fractionation of four fungal cultures of G100 and three fungal cultures of G102 grown on rice, each weighing about 25 g, were performed as described previously.³ The organic defatted extract of G102 fungal culture (240 mg) was dissolved in CHCl_3 , adsorbed onto Celite 545, and fractionated via normal-phase flash chromatography using a gradient solvent system of hexane- CHCl_3 -MeOH at a 30 mL/min flow rate and 61.0 column volumes over 34.1 min to afford five fractions. Fraction 2 (103.0 mg) was subjected to preparative HPLC using a gradient system of 40:60 to 80:20 of CH_3CN - H_2O (0.1% formic acid) over 15 min at a flow rate of 21.24 mL/min to yield 6 sub-fractions. Sub-fractions 2 and 3 yielded compounds **1** (27.8 mg) and **14** (2.3 mg), which eluted at 11.2 min and 12.7 min, respectively. Fraction 3 (26.8 mg) was subjected to preparative HPLC using a gradient system of 30:70 to 80:20 of CH_3CN - H_2O (0.1% formic acid) over 30 min at a flow rate of 21.24 mL/min to yield 10 sub-fractions. Sub-fractions 1, 2, 3, 4, 5, and 7 yielded compounds **5** (0.5 mg), **15** (0.6 mg), **16** (1.3 mg), **7** (2.8 mg), **10** (0.7 mg), and **13** (7.4 mg), which eluted at 5.5 min, 6.6 min, 8.2 min, 11.0 min, 13.5 min, and 25.7 min, respectively. Compounds **7** (2.8 mg) and **16** (1.3 mg) were subjected to further purification using semipreparative HPLC using a gradient system of 40:60 to 60:40 of CH_3OH - H_2O (0.1% formic acid) over 15 min at a flow rate of 4.72 mL/min to yield compounds **7** (0.7 mg) and **16** (1.0 mg), which eluted at 18.5 min and 12.6 min, respectively. Fraction 4 (37.5 mg) was subjected to preparative HPLC using a gradient system of 20:80 to 80:20 of CH_3CN - H_2O (0.1% formic acid) over 30 min at a flow rate of 21.24 mL/min to yield 12 sub-fractions. Sub-fractions 1, 2, 6, 7, and 8 yielded compounds **8** (2.4 mg), **11** (1.0 mg), **12** (0.9 mg), **6** (1.2 mg), and **4** (1.7 mg), which eluted at 4.3 min, 5.0 min, 13.3 min, 15.5 min, and 16.5 min, respectively. Compound **11** (1.0 mg) was subjected to further purification using semipreparative HPLC using a gradient system of 20:80 to 40:60 of CH_3OH - H_2O (0.1% formic acid) over 15 min at a flow rate of 4.72 mL/min to yield compound **11** (0.5 mg), which eluted at 13.0 min. Sub-fractions 3 (7.4 mg) and 4 (2.0 mg) were combined and subjected to preparative HPLC using an isocratic system of 20:80 of CH_3CN - H_2O (0.1% formic acid) at a flow rate of 21.24 mL/min to yield compounds **3** (0.7 mg), **9** (4.7 mg), and **2** (2.0 mg), which eluted at 43.0 min, 46.5 min, and 53.5 min, respectively. Compound **2** (2.0 mg) was subjected to further purification using semipreparative HPLC using an isocratic system of 25:75 of CH_3OH - H_2O (0.1% formic acid) at a flow rate of 4.72 mL/min to yield compound **2** (0.8 mg), which eluted at 38.0 min. Fraction 5 (63.9 mg) was subjected to preparative HPLC using a gradient system of 20:80 to 80:20 of CH_3CN - H_2O (0.1% formic acid) over 30 min at a flow rate of 21.24 mL/min to yield 9 sub-fractions. Sub-fractions 1, 3, and 5 yielded compounds **9** (2.2 mg), **17** (1.4 mg), and **18** (1.6 mg), which eluted at 7.90 min, 22.40 min, 24.30 min, respectively.

In an analogous manner, the organic defatted extract of G100 fungal culture (170 mg) was dissolved in CHCl_3 , adsorbed onto Celite 545, and fractionated via normal-phase flash chromatography using a gradient solvent system of hexane- CHCl_3 -MeOH at a 30 mL/min flow rate and 61.0 column volumes over 34.1 min to afford five fractions. Extensive preparative and semipreparative HPLC purifications of fractions 2-5 yielded compounds **1** (6.1 mg), **4** (0.8 mg), **6** (2.0 mg), **7** (1.7 mg), **8** (2.5 mg), **17** (4.6 mg), and **18** (10.7 mg).

4.3.1. Phomopsinone A (1)

Colorless solid; $[\alpha]_{\text{D}}^{24} = +121^\circ$ ($c = 0.18$, MeOH); CD ($c = 2.23 \times 10^{-4}$ M, CH_3CN) λ ($\Delta\epsilon$) 287 (+14.9) nm, 264 (+10.7) nm, 237

(+10.4) nm, 210 (−49.7) nm; HRESIMS m/z 225.1118 $[M + H]^+$ (calcd for $C_{12}H_{17}O_4$ 225.1121).

4.3.2. Phomopsinone B (2)

Colorless solid; $[\alpha]_D^{24} = +28.3^0$ ($c = 0.09$, MeOH); HRESIMS m/z 241.1070 $[M + H]^+$ (calcd for $C_{12}H_{17}O_5$ 241.1071).

4.3.3. Phomopsinone C (3)

Colorless solid; $[\alpha]_D^{24} = +42.9^0$ ($c = 0.06$, MeOH); CD (c 5.83 $\times 10^{-4}$ M, CH_3CN) λ ($\Delta\epsilon$) 294 (+7.5) nm, 271 (+6.8) nm, 250 (+7.1) nm, 214 (−13.4) nm, 207 (+16.5) nm, 200 (−24.5) nm; HRESIMS m/z 241.1070 $[M + H]^+$ (calcd for $C_{12}H_{17}O_5$ 241.1071).

4.3.4. Pyrenocine K (5)

Colorless solid; $[\alpha]_D^{24} = +43.8^0$ ($c = 0.05$, MeOH); CD (c 2.52 $\times 10^{-4}$ M, CH_3OH) λ ($\Delta\epsilon$) 300 (4.5) nm, 272 (8.5) nm, 246 (5.7) nm, 230 (12.1) nm, 223 (27.2) nm; 1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; HRESIMS m/z 239.0915 $[M + H]^+$ (calcd for $C_{12}H_{15}O_5$ 239.0914).

4.3.5. 6-Hydroxy-7-*epi*-phomopsinone A (6)

Colorless solid; $[\alpha]_D^{24} = +56.4^0$ ($c = 0.08$, MeOH); UV (MeOH) λ_{max} (log ϵ) 285 (3.41), 212 (3.28) nm; CD (c 1.25 $\times 10^{-4}$ M, CH_3CN) λ ($\Delta\epsilon$) 209 (−67.4) nm, 225 (−10.1) nm, 233 (−8.4) nm, 282 (+20.8) nm; 1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; HRESIMS m/z 241.1071 $[M + H]^+$ (calcd for $C_{12}H_{17}O_5$ 241.1071).

4.3.6. 5-Deoxy-7-hydroxypyrenocine M (7)

Colorless solid; $[\alpha]_D^{24} = -38.4^0$ ($c = 0.11$, MeOH); UV (MeOH) λ_{max} (log ϵ) 285 (3.25), 212 (3.22) nm; 1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; HRESIMS m/z 227.1278 $[M + H]^+$ (calcd for $C_{12}H_{19}O_4$ 227.1278).

4.3.7. Pyrenocine P (8)

Colorless solid; $[\alpha]_D^{24} = +78.2^0$ ($c = 0.11$, MeOH); UV (MeOH) λ_{max} (log ϵ) 282 (3.43), 214 (3.25) nm; 1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; HRESIMS m/z 213.0756 $[M + H]^+$ (calcd for $C_{10}H_{13}O_5$ 213.0758).

4.3.8. (±)-7-Hydroxypyrenocine M (9)

Colorless solid; UV (MeOH) λ_{max} (log ϵ) 283 (3.36), 213 (3.33) nm; 1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 2 and 3; HRESIMS m/z 243.1229 $[M + H]^+$ (calcd for $C_{12}H_{19}O_5$ 243.1227).

4.3.9. (±)-Pyrenocine Q (10)

Colorless solid; UV (MeOH) λ_{max} (log ϵ) 246 (3.25), 212 (3.08) nm; 1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 175 MHz), see Tables 2 and 3; HRESIMS m/z 227.1275 $[M + H]^+$ (calcd for $C_{12}H_{19}O_4$ 227.1278).

4.3.10. Pyrenocine R (11)

Colorless solid; UV (MeOH) λ_{max} (log ϵ) 284 (2.93), 211 (3.04) nm; 1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 2 and 3; HRESIMS m/z 171.0650 $[M + H]^+$ (calcd for $C_8H_{11}O_4$ 171.0652).

4.3.11. 5-Hydroxyphomopsinone A (12)

Colorless solid; $[\alpha]_D^{24} = -4.5^0$ ($c = 0.07$, MeOH); UV (MeOH) λ_{max} (log ϵ) 280 (3.45), 213 (3.28) nm; 1H NMR ($CDCl_3$, 500

MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 2 and 3; HRESIMS m/z 241.1071 $[M + H]^+$ (calcd for $C_{12}H_{17}O_5$ 241.1070).

4.3.12. 8-Hydroxyblennolide H (14)

Colorless solid; $[\alpha]_D^{24} = -113.3^0$ ($c = 0.11$, MeOH); UV (MeOH) λ_{max} (log ϵ) 334 (3.26), 277 (3.36), 222 (3.46) nm; CD (c 1.56 $\times 10^{-4}$ M, $CHCl_3$) λ ($\Delta\epsilon$) 379 (−14.4) nm, 328 (−23.4) nm, 293 (−19.4), 248 (−43.3), 236 (+52.1), 226 (−126.2) nm; 1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 4; HRESIMS m/z 321.0968 $[M + H]^+$ (calcd for $C_{16}H_{17}O_7$ 321.0969).

4.3.13. *cis*-Dihydro-8-hydroxyblennolide H (15)

Colorless oil; $[\alpha]_D^{24} = -72.7^0$ ($c = 0.09$, MeOH); UV (MeOH) λ_{max} (log ϵ) 354 (3.18), 276 (3.55), 222 (3.53) nm; CD (c 1.86 $\times 10^{-4}$ M, $CHCl_3$) λ ($\Delta\epsilon$) 344 (−2.2) nm, 274 (+45.0) nm, 237 (+87.1) nm, 225 (−113.6) nm; 1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 4; HRESIMS m/z 323.1117 $[M + H]^+$ (calcd for $C_{16}H_{19}O_7$ 323.1125).

4.3.14. *trans*-Dihydro-8-hydroxyblennolide H (16)

Colorless oil; $[\alpha]_D^{24} = +38.2^0$ ($c = 0.08$, MeOH); UV (MeOH) λ_{max} (log ϵ) 349 (3.13), 272 (3.48), 222 (3.48) nm; CD (c 2.17 $\times 10^{-4}$ M, $CHCl_3$) λ ($\Delta\epsilon$) 344 (+6.9) nm, 274 (−54.9) nm, 237 (−18.4) nm, 225 (+22.1) nm; 1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 4; HRESIMS m/z 323.1126 $[M + H]^+$ (calcd for $C_{16}H_{19}O_7$ 323.1125).

4.3.15. Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of phomopsinone B (2), phomopsinone C (3), 6-hydroxy-7-*epi*-phomopsinone A (6) and 5-deoxy-7-hydroxypyrenocine (7).

To 0.13, 0.15, 0.2 and 0.15 mg of compounds **2**, **3**, **6** and **7** were added 400 μ L of pyridine- d_5 and transferred into NMR tubes. To initiate the reactions, 10 μ L of *S*-(+)- α -methoxy- α -(trifluoromethyl)phenylacetyl (MTPA) chloride were added into each tube with careful shaking and then monitored immediately by 1H NMR at the following time points 5, 10, 15, and 30 min. The reactions completed within 5, 5, 30 and 5 min, respectively, yielding the mono (*R*)-MTPA ester derivatives (**2b**) of **2**, (**3b**) of **3**, (**6b**) of **6**, and (**7b**) of **7**. 1H NMR data (500 MHz, pyridine- d_5) of **2b**: δ_H 4.31 (1H, d, $J = 14.9$, H-5a), 4.61 (1H, d, $J = 14.9$, H-5b), 1.83 (2H, m, H-8), 5.62 (1H, m, H-9), 1.30 (3H, d, $J = 5.7$, H-10); of **3b**: δ_H 4.15 (1H, d, $J = 14.9$, H-5a), 4.52 (1H, d, $J = 14.9$, H-5b), 2.08 (1H, m, H-6a), 2.33 (1H, m, H-6b), 1.75 (1H, m, H-8a), 5.52 (1H, m, H-9), 1.38 (3H, d, $J = 6.3$, H-10); of **6b**: δ_H 5.68 (1H, s, H-3), 4.32 (1H, d, $J = 14.9$, H-5a), 4.49 (1H, d, $J = 14.9$, H-5b), 5.99 (1H, d, $J = 6.9$, H-6), 1.45 (1H, m, H-9a), and 0.88 (3H, t, $J = 7.5$, H-10); of **7b**: 5.79 (1H, s, H-3), 1.84 (3H, s, H-5), 2.82 (1H, dd, $J = 14.9$, 5.2, H-6a), 3.03 (1H, dd, $J = 14.9$, 8.6, H-6b), 5.64 (1H, m, H-7), 1.60 (1H, m, H-8a), 1.69 (1H, m, H-8b), 1.23 (1H, m, H-9a), and 0.76 (3H, t, $J = 7.5$, H-10). In an analogous manner, 0.13, 0.15, 0.2 and 0.15 mg of **2**, **3**, **6** and **7** dissolved in 400 μ L pyridine- d_5 were reacted in NMR tubes with 10 μ L (*R*)-(-)- α -MTPA chloride for 30 and 5 min, respectively, to afford the mono (*S*)-MTPA esters (**2a**, **3a**, **6a** and **7a**). 1H NMR data (500 MHz, pyridine- d_5) of **2a**: δ_H 4.21 (1H, d, $J = 14.9$, H-5a), 4.57 (1H, d, $J = 14.9$, H-5b), 1.79 (2H, m, H-8), 5.60 (1H, m, H-9), 1.37 (3H, d, $J = 6.3$, H-10); of **3a**: δ_H 4.26 (1H, d, $J = 14.9$, H-5a), 4.57 (1H, d, $J = 14.9$, H-5b), 2.14 (1H, m, H-6a), 2.44 (1H, m, H-6b), 1.79 (1H, m, H-8a), 5.59 (1H, m, H-9), 1.29 (3H, d, $J = 6.3$, H-10); of **6a**: δ_H 5.74 (1H, s, H-3), 4.30 (1H, d, $J = 14.9$, H-5a), 4.51 (1H, d, $J = 14.9$, H-5b), 6.03 (1H, d, $J = 6.9$, H-6), 1.35 (1H, m, H-9a), and 0.83 (3H, t, $J = 7.5$, H-10); of **7a**:

5.73 (1H, s, H-3), 1.62 (3H, s, H₃-5), 2.70 (1H, dd, $J = 14.9, 4.0$, H-6a), 2.96 (1H, dd, $J = 14.9, 8.6$, H-6b), 5.66 (1H, m, H-7), 1.64 (1H, m, H-8a), 1.76 (1H, m, H-8b), 1.35 (1H, m, H-9a), and 0.83 (3H, t, $J = 7.5$, H₃-10).

4.4. X-ray crystallography

Crystallographic data for compound **8** has been deposited with the Cambridge Crystallographic Data Centre, deposition number CCDC 1473567. Compound's **8** crystals were grown in MeOH at rt. X-ray crystal structure analysis of **8** were as follows: formula C₁₀H₁₂O₅, MW = 212.20, irregular, colourless crystal, approximate dimensions 0.28 × 0.24 × 0.13 mm, $a = 4.1747$ (3) Å, $b = 10.9965$ (7) Å, $c = 11.0671$ (7) Å, $\alpha = 70.466$ (1)°, $\beta = 85.814$ (1)°, $\gamma = 80.816$ (1)°, $T = 193$ K, $Z = 2$, triclinic $P1$, $GOF = S = 1.03$, $V = 472.57$ (5) Å³, R_{int} (5123 reflections, $I > 2\sigma(I)$) = 0.019, $wR(F^2) = 0.099$, $\lambda = 0.71073$ Å. Crystal data, data collection, structure solution and refinement details are summarized in Table S2.

4.5. Bioassay methods

Pth1 from *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* was expressed and purified as described previously.^{40,41} Inhibition was determined using established procedures.^{28,30} Briefly quantification of bulk peptidyl-tRNA cleavage to free tRNA was quantified using electrophoretic separation via acid-urea gel to determine inhibitory activity of compounds under study.

Acknowledgments

The authors from the University of North Carolina at Greensboro were supported in part by internal research and development funds. We thank the City of Greensboro for permission to collect in Lake Brandt. The high-resolution mass spectrometry data were acquired in the Triad Mass Spectrometry Laboratory at the University of North Carolina at Greensboro. The Wake Forest University X-ray Facility thanks the National Science Foundation (grant CHE-0234489) for funds to purchase the X-ray instrument and computers. We thank Dr. A. Horswill of the University of Iowa for helpful discussions, and Dr. J. O. Falkinham III of the Virginia Polytechnic Institute and State University for antimicrobial testing.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at.....

References and notes

- Raja, H. A., Oberlies, N. H., El-Elimat, T., Miller, A. N., Zelski, S. E., Shearer, C. A. *Mycoscience* **2013**, *54*, 353.
- Raja, H. A., Oberlies, N. H., Figueroa, M., Tanaka, K., Hirayama, K., Hashimoto, A., Miller, A. N., Zelski, S. E., Shearer, C. A. *Mycologia* **2013**, *105*, 959.
- El-Elimat, T., Raja, H. A., Figueroa, M., Falkinham III, J. O., Oberlies, N. H. *Phytochemistry* **2014**, *104*, 114.
- El-Elimat, T., Raja, H. A., Day, C. S., Chen, W.-L., Swanson, S. M., Oberlies, N. H. *J. Nat. Prod.* **2014**, *77*, 2088.
- Alali, F. Q., Amrine, C. S. M., El-Elimat, T., Alkofahi, A., Tawaha, K., Gharaibah, M., Swanson, S. M., Falkinham III, J. O., Cabeza, M., Sánchez, A., Figueroa, M., Oberlies, N. H. *Phytochem. Lett.* **2014**, *9*, 96.
- Shearer, C. A., Descals, E., Kohlmeyer, B., Kohlmeyer, J., Marvanova, L., Padgett, D., Porter, D., Raja, H. A., Schmit, J. P., Thorton, H. A., Voglymayr, H. *Biodivers. Conserv.* **2007**, *16*, 49.
- Hernández-Carlos, B., Gamboa-Angulo, M. *Phytochem. Rev.* **2011**, *10*, 261.
- Dong, J. Y., Shen, K. Z., Sun, R. *Mycosystema* **2011**, *30*, 206.
- Hussain, H., Ahmed, I., Schulz, B., Draeger, S., Krohn, K. *Fitoterapia* **2012**, *83*, 523.
- Hussain, H., Krohn, K., Ahmed, I., Draeger, S., Schulz, B., Pietro, S., Pescitelli, G. *Eur. J. Org. Chem.* **2012**, 1783.
- Bräse, S., Encinas, A., Keck, J., Nising, C. F. *Chem. Rev.* **2009**, *109*, 3903.
- Masters, K.-S., Bräse, S. *Chem. Rev.* **2012**, *112*, 3717.
- Stoll, A., Renz, J., Brack, A. *Helv. Chim. Acta* **1952**, *35*, 2022.
- Kurobane, I., Iwahashi, S., Fukuda, A. *Drugs Exp. Clin. Res.* **1987**, *13*, 339.
- Liao, G., Zhou, J., Wang, H., Mao, Z., Xiao, W., Wang, H., She, Z., Zhu, Y. *Oncol. Rep.* **2010**, *23*, 387.
- McPhee, F., Caldera, P. S., Bemis, G. W., McDonagh, A. F., Kuntz, I. D., Craik, C. S. *Biochem. J.* **1996**, *320* (Pt 2), 681.
- El-Elimat, T., Figueroa, M., Raja, H. A., Graf, T. N., Swanson, S. M., Falkinham III, J. O., Wani, M. C., Pearce, C. J., Oberlies, N. H. *Eur. J. Org. Chem.* **2014**, *2015*, 109.
- Hegde, V. R., Puar, M. S., Dai, P., Pu, H., Patel, M., Anthes, J. C., Richard, C., Terracciano, J., Das, P. R., Gullo, V. *J. Antibiot.* **2001**, *54*, 125.
- McFeeters, R. L. *JSM Biotechnol. Bioeng* **2013**, *1*, 1006.
- Hames, C. M., McFeeters, H., Holloway, B. W., Stanley, B. C., Urban, S. V., McFeeters, L. R. *Int. J. Mol. Sci.* **2013**, *14*, 22741.
- Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., Fungal Barcoding Consortium. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 6241.
- Hoye, T. R., Jeffrey, C. S., Shao, F. *Nat. Protoc.* **2007**, *2*, 2451.
- Franck, B., Gottschalk, E.-M., Ohnsorge, U., Hüper, F. *Chem. Ber.* **1966**, *99*, 3842.
- Qin, T., Iwata, T., Ransom, T. T., Beutler, J. A., Porco, J. A. *J. Am. Chem. Soc.* **2015**, *137*, 15225.
- Zhang, W., Krohn, K., Ullah, Z., Florke, U., Pescitelli, G., Di Bari, L., Antus, S., Kurtan, T., Rheinheimer, J., Draeger, S., Schulz, B. *Chem. - Eur. J.* **2008**, *14*, 4913.
- Isaka, M., Palasarn, S., Komwijit, S., Somrithipol, S., Sommai, S. *Tetrahedron Lett.* **2014**, *55*, 469.
- Harris, S. M., McFeeters, H., Ogungbe, I. V., Cruz-Vera, L. R., Setzer, W. N., Jackes, B. R., McFeeters, R. L. *Nat. Prod. Commun.* **2011**, *6*, 1421.
- McFeeters, H., Gilbert, M. J., Thompson, R. M., Setzer, W. N., Cruz-Vera, L. R., McFeeters, R. L. *Nat. Prod. Commun.* **2012**, *7*, 1107.
- Ferguson, P. P., Holloway, B. W., Setzer, N. W., McFeeters, H., McFeeters, L. R. *Antibiotics* **2016**, *5*.
- Holloway, W. B., McFeeters, H., Powell, A. M., Nidadavolu, G. S., McFeeters, R. L. *J. Anal. Bioanal. Tech.* **2015**, *6*, 244.
- Payne, D. J., Gwynn, M. N., Holmes, D. J., Pompliano, D. L. *Nat. Rev. Drug Discovery* **2007**, *6*, 29.
- Shearer, C. A., Langsam, D. M., Longcore, J. E., 2004. Fungi in Freshwater Habitats. In: Mueller, G. M., Bills, G. F., Foster, M. S. (Eds.), Measuring and Monitoring Biological Diversity: Standard Methods for Fungi. Smithsonian Institution Press, Washington, D.C., pp. 513.
- Raja, H. A., Shearer, C. A. *Mycologia* **2008**, *100*, 467.
- Raja, H., Schmit, J., Shearer, C. *Biodivers. Conserv.* **2009**, *18*, 419.
- El-Elimat, T., Figueroa, M., Raja, H. A., Adcock, A. F., Kroll, D. J., Swanson, S. M., Wani, M. C., Pearce, C. J., Oberlies, N. H. *Tetrahedron Lett.* **2013**, *54*, 4300.

36. Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., Bates, S. T., Bruns, T. D., Bengtsson-Palme, J., Callaghan, T. M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G. W., Hartmann, M., Kirk, P. M., Kohout, P., Larsson, E., Lindahl, B. D., Lücking, R., Martín, M. P., Matheny, P. B., Nguyen, N. H., Niskanen, T., Oja, J., Peay, K. G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J. A., Senés, C., Smith, M. E., Suija, A., Taylor, D. L., Telleria, M. T., Weiss, M., Larsson, K.-H. *Mol. Ecol.* **2013**, 22, 5271.
37. Zhang, H., Hyde, K. D., McKenzie, E. H. C., Bahkali, A. H., Zhou, D. *Cryptogamie, Mycologie* **2012**, 33, 333.
38. Hyde, K. *Aust. Syst. Bot.* **1993**, 6, 169.
39. Schmitt, I., Barker, F. K. *Nat. Prod. Rep.* **2009**, 26, 1585.
40. Vandavasi, V., Taylor-Creel, K., McFeeters, R. L., Coates, L., McFeeters, H. *Acta Crystallogr., Sect. F: Struct. Biol. Commun.* **2014**, 70, 872.
41. Hughes, R. C., McFeeters, H., Coates, L., McFeeters, R. L. *Acta Crystallogr. Sect. F* **2012**, 68, 1472.

Graphical Abstract

To create your abstract, type over the instructions in the template box

α -Pyrone derivatives, tetra/hexahydroxanthones, and cyclodepsipeptides from two freshwater fungi

Tamam El-Elimat^a, Huzefa A. Raja^b, Cynthia S. Day^c, Hana McFeeters^d, Robert L. McFeeters^d, and Nicholas H. Oberlies^{b,*}

Leave this area blank for abstract info.

^a Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid 22110, Jordan

^b Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, North Carolina 27402, United States

^c Department of Chemistry, Wake Forest University, Winston–Salem, North Carolina 27109, United States

^d Department of Chemistry, University of Alabama in Huntsville, Huntsville, Alabama 35899, United States